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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Dean Pettit and Claudia M. Jochheim
Application No.	:	09/800,016
Filed	:	March 5, 2001
For	:	USE OF EDTA IN STABILIZING GRANULOCYTE MACROPHAGE COLONY-STIMULATING FACTOR (As Amended)

Examiner	:	Lorraine Spector, Ph.D.
Art Unit	:	1647
Docket No.	:	140145.410
Date	:	February 26, 2007

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, Andreas Baumann, declare:

1. I am the Department Head of Biologicals, Function Pharmacokinetics, Corporate Preclinical Development, at Schering AG (the assignee of the present application, now Bayer Schering Pharma Aktiengesellschaft, and my curriculum vitae is attached as Exhibit 1.

2. I have reviewed the Office Action dated September 28, 2006, in the subject application, including the rejections under 35 U.S.C. 103(a), and provide this Declaration to assist the Examiner in evaluating the non-obviousness of the claimed subject matter of the application.

3. The studies presented herein were performed by me, by those under my direct supervision, or are within my personal knowledge.

4. Methods and Materials: The pharmacokinetic profiles of three sargramostim formulations after single subcutaneous administration in Cynomolgus monkeys were compared. The three sargramostim formulations are as follows: (1) liquid formulation with EDTA and benzyl alcohol: 500 µg/ml sargramostim, 5.5 mM EDTA, 1.15% benzyl alcohol, 40 mg/ml mannitol, 10 mg/ml sucrose, and 10 mM Tris-HCl (pH 6.7 to 7.7); (2) lyophilized material dissolved in benzyl alcohol but without EDTA: 500 µg/ml sargramostim, 0.9% benzyl alcohol, 40 mg/ml mannitol or 10 mg/ml sucrose, and 10 mM Tris-HCl, pH 6.15; and (3) liquid formulation without EDTA or benzyl alcohol: 500 µg/ml sargramostim and 100 mM Tris-HCl, pH 7.4.

More specifically, six female animals per group and formulation received a single subcutaneous dose of 20 µg/kg sargramostim. Blood samples were obtained 0, 5, 15, and 30 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 h post drug administration and processed to serum. In the group that was administered with the liquid formulation without EDTA or benzyl alcohol, additionally at 32 and 48 h, post dose blood samples were withdrawn.

Serum samples were analyzed for sargramostim concentration using a validated ELISA method as described below. Briefly, a "sandwich" ELISA assay design based on R&D Systems high sensitivity human GM-CSF ELISA immunoassay was used. The assay employed a quantitative sandwich enzyme immunoassay technique. Ninety-six well microtiter plates were pre-coated by the manufacturer with a murine monoclonal antibody specific for GM-CSF. After preparation, sargramostim calibrators, sargramostim controls, and samples were pipetted into the microtiter plate wells and incubated for approximately 3 h at room temperature to permit the (soluble) sargramostim to bind to the immobilized anti-GM-CSF. The plate was washed to remove any unbound substances. Alkaline phosphatase linked to a murine monoclonal antibody against GM-CSF was added to the wells and incubated for approximately 2 h at room temperature allowing the (bound) sargramostim to be sandwiched between the

immobilized antibody and the conjugated antibody. Following a wash to remove any unbound conjugate, a NADPH substrate was added to the wells and incubated for approximately 1 h at room temperature. An amplifier solution was subsequently added and incubated for approximate 30 min. at room temperature. The enzyme substrate reaction was quenched with sulfuric acid and the absorbance was measured at 490-650 nm in a microtiter plate reader. The intensity of the color produced (absorbance) is directly proportional to the concentration of sargramostim present. The lower limit of quantification (LLOQ) was 2 pg/ml.

Pharmacokinetic parameters were calculated from individual serum concentration time profiles of 6 female monkeys per formulation using the software KINETICA, version 4.3 (Thermo Corp., USA) according to Table 1:

Table 1: Pharmacokinetic parameters, their symbol and way of calculation for pharmacokinetic evaluation

Parameter	Symbol	Way of calculation
Maximum concentration	C_{max}	Taken as directly determined from the serum concentration – time data
Sampling time of C_{max}	t_{max}	Taken as directly determined from the serum concentration – time data
Last sampling time with a valid concentration value >LLOQ	t_{last}	Taken as directly determined from the serum concentration-time profiles
Terminal half-life	$t_{1/2}$	$t_{1/2} = \ln 2/\lambda_z$
Area under the concentration-time curve from zero to 2 h	AUC(0-2h)	Non-compartmental analysis applying the mixed linear/logarithmic trapezoidal rule; extrapolation of the area from the first data point to the time of administration by linear connection of the first data point with the origin ($t_0;C_0 = 0;0$)
Area under the concentration-time curve from zero to 4 h	AUC(0-4h)	Non-compartmental analysis applying the mixed linear/logarithmic trapezoidal rule; extrapolation of the area from the first data point to the time of administration by linear connection of the first data point with the origin ($t_0;C_0 = 0;0$)
Area under the concentration-time curve from zero to t_{last}	AUC(0-t_{last})	Like $AUC_{(0-24h)}$, but from time of administration until last sampling time with a valid concentration value > LLOQ (t_{last})
Area under the concentration-time curve from zero to infinity	AUC	$AUC = AUC_{(0-tlast)} + C_{tlast}/\lambda_z$ (C_{tlast} = concentration value at t_{last})
Mean residence time	MRT	MRT = AUMC/ AUC

Values below the lower limit of quantification (LLOQ) were set to zero.

When appropriate, results are summarized as mean ± SD (standard deviation). In addition, the coefficient of variation [%] was calculated.

5. Results: The individual maximum serum concentrations (C_{max}) after administration of the liquid formulation with EDTA and benzyl alcohol were observed at different time points (Exhibit 2). In addition, the mean maximum serum concentrations (C_{max}) at different time points as well as other mean pharmacokinetic parameters after single subcutaneous administration of the three different formulations were obtained (Exhibit 3 and Table 2). The results show that after application of the liquid formulation with EDTA and benzyl alcohol, a double peak occurred, which was not seen after administration of the two other formulations. The first peak was observed with a steep concentration increase very shortly after dosing indicating rapid absorption of a first part of sargramostim from the formulation. The second peak was observed in the same time period of the t_{max} of sargramostim after application of the two other formulations (1-3 h post dose).

Resulting from the first peak after application of the liquid formulation with EDTA and benzyl alcohol, the initial systemic exposure until one hour post dose (Exhibit 3) is much higher compared to the liquid formulation without EDTA and with benzyl alcohol and also higher in comparison with the third formulation (*i.e.*, the formulation without EDTA or benzyl alcohol).

Table 2: Mean (± SD) pharmacokinetic parameters after single subcutaneous administration of three different formulations of 20 µg/kg sargramostim to female monkeys (n= 6/ group)

Parameter	Unit	Liquid (with EDTA and benzyl alcohol)	Lyophilized material dissolved in benzyl alcohol (without EDTA)	Liquid (without EDTA or benzyl alcohol)
C_{max}	[ng/ml]	12.3 ± 3.03	9.15 ± 2.67	13.2 ± 1.77
t_{max}	[h]	0.417 ± 0.303 (0.250) [0.250/1.00]*	2.75 ± 0.418 (3.00) [2.00/3.00]*	1.67 ± 0.516 (1.50) [1.00/ 2.50]*
$t_{1/2}$	[h]	2.73 ± 0.457	2.38 ± 0.300	2.12 ± 0.399
AUC(0-2h)	[ng/ml x h]	19.4 ± 4.79	10.7 ± 3.22	18.2 ± 2.81
AUC(0-4h)	[ng/ml x h]	36.3 ± 11.0	27.2 ± 7.11	37.4 ± 3.68
AUC(0-t_{last})	[ng/ml x h]	65.4 ± 13.8	53.8 ± 15.9	55.4 ± 7.83
MRT	[h]	4.76 ± 1.21	4.92 ± 0.544	3.34 ± 0.324
t_{last}	[h]	20.0 ± 6.20 (24.0) [12.0/24.0]*	22.0 ± 4.90 (24.0) [12/24]*	23.3 ± 6.41 (24.0) [12.0/ 24.0]*

C_{max}	Maximum measured concentration of drug in serum post dose
t_{max}	Time to reach C_{max}
t_{last}	Last sampling time with a valid concentration value > LLOQ of 2.0 pg/ml
$t_{1/2}$	Terminal half-life
AUC(0-xh)	Area under the concentration versus time curve from zero to x h
AUC(0-t_{last})	Area under the concentration versus time curve from dosing time to t_{last}
MRT	Mean residence time AUMC/ AUC
*	(median) [min. value/ max. value]

Major differences could be observed in the pharmacokinetic profile (absorption time, C_{max}) as well as in the exposure (AUC) after administration of the sargramostim liquid formulation with EDTA and benzyl alcohol and the sargramostim lyophilized material dissolved in benzyl alcohol but without EDTA. The sargramostim liquid formulation with EDTA and benzyl alcohol was more rapidly absorbed as indicated by earlier t_{max} and by about 30% higher C_{max}-values. The AUC after administration of the liquid formulation exceeds that of the sargramostim lyophilized material dissolved in benzyl alcohol but without EDTA for the partial AUC's 0-2 h, 0-4 h and 0-24 h by 80, 40 and 20%, respectively.

The influence of the formulation ingredients EDTA and/or benzyl-alcohol on the pharmacokinetic behavior of sargramostim was investigated by single dose s.c.

administration of a sargramostim liquid formulation without EDTA or benzyl alcohol (sargramostim re-constituted in Tris buffer). The absorption behavior of this formulation with t_{max}-values of about 1.7 h was more similar to the sargramostim lyophilized material dissolved in benzyl alcohol but without EDTA than to the sargramostim liquid formulation with EDTA and benzyl alcohol. No double peak was observed likewise. However, C_{max}-values were similar to those observed after administration of the sargramostim liquid formulation with EDTA and benzyl alcohol. Nevertheless, the total AUC-values were again comparable to the sargramostim lyophilized material dissolved in benzyl alcohol but without EDTA.

These results indicate that the double-peak absorption profile is unique to the sargramostim formulation with EDTA after subcutaneous administration. EDTA induces initial rapid absorption of sargramostim to cause the initial intravenous-like absorption behavior of the sargramostim liquid formulation with EDTA and benzyl alcohol. After expenditure of EDTA, the absorption behavior comes back to the "normal" subcutaneous manner represented by the second more gradual peak of the serum level time curve.

6. The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Prof. Dr. Andreas Baumann

26. March 2007
Date

EXHIBIT 1

Dr. Baumann's Curriculm Vitae

1. Education

2005	Professorship in Pharmacology & Toxicology Medical Faculty, University Greifswald, Germany
1992	Habilitation (Higher Doctorate) University Greifswald, Habilitation thesis: " Metabolism studies for risk assessment in drug development ", Venia legendi in Pharmacology /Toxicology
1985	Doctorate ("Summa cum laude") University Greifswald, Doctoral thesis: " Metabolism of a new ß-blocking agent in rats"
1982-86	Postgraduate study and title Toxicology specialist University Berlin
1980	Certification as state-registered pharmacist incl. Pharmacology/toxicology
1980	Masters degree in pharmacy and pharmacology/toxicology University Greifswald, Dept. of Pharmacology

2. Career

2001-present	Schering AG, Berlin, Department Head, Function Pharmacokinetics/ Biologicals, Corporate Preclinical Development
1998 - 2001	Schering AG, Berlin, Head of the Department of Research Pharmacokinetics, Drug Research
1991-1998	Schering AG, Berlin, Lab. Leader Pharmacokinetics, Biological Development, Fertility control and hormon therapy
1986	Received "Rudolf-Buchheim-Award" of the German Society of Pharmacology and Toxicology
1985 -present	Lecturing at University Greifswald
1980 - 1991	Research Scientist, University Greifswald

3. Scientific publications

A: Articles

1. D. Mould, A. Baumann, M.J. Keating, P. Bonate

Population Pharmacokinetics-Pharmacodynamics of Alemutzumab in patients with Chronic Lymphocytic Leukemia

BJCP (British J. Clin. Pharmacol.), (2007) in press

2. A. Baumann

Early development of therapeutic biologics – Pharmacokinetics

Current Drug Metabolism, **7**, (2006), 15 -21

3. D. Högemann, A. Baumann, D. Rocker, A. Bader, M.Galanski

In vitro model of the human liver parenchyma to study hepatotoxic side effects of Dy-EOB-DTPA

Investigative Radiology, (2000), 373-379

4. A. Baumann, W. Feser, P. Cramer, R.S. Kerdar, H. Blode, J. Körber, W. Kuhnz

Use of precision-cut human liver slices for studying the metabolism and genotoxic potency of xenobiotics by means of the ^{32}P-postlabeling technique: Steps toward method validation using testosterone and 2-aminofluorene

Biomarkers, **4**, (1999), 188-202

5. A. Baumann, Ch. Gansau, D. Rocker, R. Zierz, A. Salomon

Use of long-term cultured hepatocytes for assessing hepatotoxicity

ISSX-Proceedings, **13** (1998), 308

6. D. Rocker, A. Baumann, C. Gansau, R. Zierz, A. Salomon, W. Kuhnz

Is enzyme release a useful parameter to investigate cytotoxic effects of xenobiotics in human and dog hepatocyte sandwich cultures?

HUG-Letters, 7, (1997), 17-18

7. W. Feser, R.S. Kerdar, A. Baumann, J. Körber, H. Blode, W. Kuhnz
DNA-adduct formation of selected sex steroids in human liver slices in vitro
Toxicology in vitro, **12**, (1998), 353-364

8. A. Baumann, W. Feser, P. Cramer, R.S. Kerdar
Validation of precision-cut human liver slices as an in vitro-model for studying the genotoxic potency of xenobiotics by means of the ^{32}P-postlabeling technique
ISSX-Proceedings, **11** (1997), 111

9. P.H. Bach, A.E.M. Vickers, R. Fisher, A. Baumann, et. al.
The use of tissue slices for pharmacotoxicology studies
ATLA **24** (1996), 893-923

10. A. Baumann, R.S. Kerdar, P.Cramer, W. Feser, H. Blode, A. Salomon, W. Kuhnz
Use of rat and human liver slices for the detection of steroid hormone-induced DNA-adducts *in vitro* by means of the ^{32}P-postlabeling technique
Pharmacology & Toxicology, **78**, (1996), 214 – 223

11. A. Baumann, H. Kulmann, V. Gorkov, M. Mahler, W. Kuhnz
Radioimmunological analysis of cyproterone acetate in human serum - Comparison with a gas chromatographic/mass spectrometric method and influence of each method on the outcome of a bioequivalence trial
Arzneim.-Forsch./Drug Res. **46**, (1996), 412 – 418

12. A. Baumann, A. Fuhrmeister, M .Brudny-Kloeppel, C. Draeger, T. Bunte, W. Kuhnz
Comparative pharmacokinetics of two new steroidal estrogens and ethinylestradiol in postmenopausal women
Contraception **54** (1996), 235-242

13. A. Baumann, R. Reimann
Use of rat hepatocytes as a suitable in vitro model to predict the in vivo distribution of steroid hormones into the liver
HUG-Letters, **6**, (1996), 17-18

14. R. S. Kerdar, A. Baumann, M. Brudny-Klöppel, H. Biere, H. Blode, W. Kuhnz

Identification of 3□-hydroxy-cyproterone acetate as a metabolite of cyproterone acetate in the bile of female rats and the potential of this and other already known or putative metabolites to form DNA adducts in *vitro*

Carcinogenesis, **16** (1995), 1835 - 1841

15. A. Baumann, R.S. Kerdar, P. Cramer, Werner Feser

Use of human liver slices to investigate the propensity of 2-Aminofluorene to induce DNA-adducts in vitro

ISSX-Proceedings, **8** (1995), 212

16. A. Baumann, R.S. Kerdar, P.Cramer, W. Feser, H. Blode, A. Salomon, W. Kuhnz

Use of human liver slices to investigate the genotoxic potency of xenobiotics by means of the ^{32}P-postlabeling technique

HUG-Letters, **5**, (1995), 17-18

17. W. Kuhnz, A. Baumann, T. Staks, L. Dibbelt, R. Knuppen, G. Jütting

Pharmacokinetics of Gestodene and Ethinylestradiol in 14 women during three month of treatment with a new tri-step combination oral contraceptive

Ob./Gyn. Digest., June (1994), 20 - 22

18. W. Kuhnz, A. Baumann, T. Staks, L. Dibbelt, R. Knuppen, G. Jütting

Pharmacokinetics of Gestodene and Ethinylestradiol in 14 women during three month of treatment with a new tri-step combination oral contraceptive

- Serum protein binding of Gestodene and influence of treatment on free and total testosterone levels in serum-

Contraception, **48** (1993), 303 - 322

19. A. Baumann, K.-U. Möritz

Untersuchungen zur kovalenten Bindung eines potentiellen ß-Rezeptorenblockers (B 24/76) an Gewebsproteine und Kollagen bei der Ratte

Pharmazie, **46** (1991), 655-657

20. M. Dittgen, H. Adam, K. Przylucki, A. Baumann

Einfluß der Lipophilie des Wirkstoffs und des Konstruktionsprinzips von Transdermalpflastern mit ß-Blockern auf die Blutspiegel am Tier

Pharmazie, **46** (1991), 608-609

21. A. Baumann

Die Bedeutung von Metabolismusstudien für die Risikobewertung von Arzneimitteln - dargestellt am Beispiel eines potentiellen ß-Rezeptorenblockers

Dissertation (habil), Ernst-Moritz-Arndt-Universität Greifswald, 1991

22. K.-U. Möritz, A. Baumann, G. Schwesinger, M. Madaus

Zur Wirkung von 2,4-Dichlorphenol auf Enzymaktivitäten und andere Parameter in parenchymatösen Organen von Ratten

Die Nahrung, **36** (1992), 273-278

23. A. Baumann

A novel metabolic pathway of a ß-blocking agent: Cyclisation of an aryloxypropanolamine to an oxazolidone derivative

ISSX-Proceedings, **3** (1993), 63

24. A. Baumann, J. Hoffmann, F. Riemer, A. Grisk

Determination of 2.4-dichlorophenol in urin and serum by hplc and gc

Pharmazie, **45** (1990), 218-219

25. A. Baumann, K. Przylucki, H. Adam, G. Franke, M. Dittgen

Pharmakokinetik von ß-Rezeptorenblockern nach transdermaler Applikation an der Ratte

Archiv der Pharmazie, **323** (1990), 783

26. M. Zschiesche, A. Baumann

Determination of the ß-adrenoceptor blocking drug B 24/76 in serum by HPLC with fluorimetric detection after precolumn dansylation

Journal of Chromatographie, **489** (1989), 482 – 486

27. A. Baumann, R. Seefeld, I. Werner, B. Seifert , A. Grisk

Biotransformation von B24/76 bei Ratte, Minischwein, Hund und Mensch

Pharmazie, **44** (1989), 215 – 218

28. A. Baumann, A. Müller, S. Pollex, B. Seifert, I. Werner, A. Grisk

Species differences in drug metabolism and its importance for toxicity - a comparative study with the ß-blocker B 24/76

Zent. bl. Pharm. Pharmakother. Lab. diagn., **127** (1988), 316

29. A. Baumann, I. Werner, B. Kelling, I. Amon, K. Janowski, A. Grisk
Metabolism of the new ß-adrenergic blocking drug B 24/76 in minipigs
Plzen lek. Sborn. , **Suppl. 53** (1986), 109 – 11

30. I. Amon, A. Baumann, B. Hartmann, K.-P. Henschel, A. Müller
Distribution of metabolites of the potent ß-adrenoceptor blocking drug B 24/76 in different tissues of rats under toxic conditiones
Arch. Toxicol., **Suppl. 8** (1985), 218

31. A. Baumann, R. Seefeld
Identifizierung von Metaboliten des potentiellen ß-Rezeptorenblockers B 24/76 mit Hilfe der Massenspektrometrie
ZfII-Mitteilungen, Leipzig, **115** (1986), 151 – 158

32. A. Baumann
Biotransformation und Pharmakokinetik von DL-1-(2,4-Dichlor-phenoxy)-3-2-(3,4-dimethoxy-phenyl)-ethylamino-propan-2-ol an der Ratte
Mit. Bl. Ges. experi. Med. DDR, **24** (1987) 51 – 52

33. A. Baumann
Biotransformation von DL-1-(2,4-Dichlor-phenoxy)-3-2-(3,4-dimethoxy-phenyl)-ethylamino-propan-2-ol an der Ratte
Thesis, Ernst-Moritz-Arndt-Universität Greifswald, 1984

34. S. Schnitzler, A. Baumann, H. Renner, H. Klein, P.G. Reitnauer
Kinetische Messung kutaner anaphylaktischer Reaktionen bei der Ratte
Z. med. Labor-Diagn., **22** (1981) 23 - 28

B: Books

1. A. Baumann

Die Clearance

in: W. Cawello (Hrsg.), Parameter zur modellunabhängigen Pharmakokinetik, 81-96, Shaker-Verlag, Aachen, 1998, ISBN3-8265-3940-0

2. A. Baumann

Concept of Clearance

in: W. Cawello (Ed.), Parameters for Compartment-free Pharmacokinetics, Standardisation of Study Design, Data Analysis and Reporting, 81-95, Shaker-Verlag, Aachen, 1999, ISBN 3-8265-4767-5

3. O. Pelkonen, A. Baumann, A. Reichel (Editors)

Pharmacokinetic Challenges in Drug Discovery, Springer, Berlin, Heidelberg, New York, 2002, ISBN 3-540-42585-3

EXHIBIT 2

Individual serum level time profiles of sargramostim (*i.e.*, ZK 250224) in female monkeys (N=6) after single subcutaneous administration of 20 µg/kg sargramostim of the liquid formulation with EDTA and benzyl alcohol over a sampling period of 24 h.

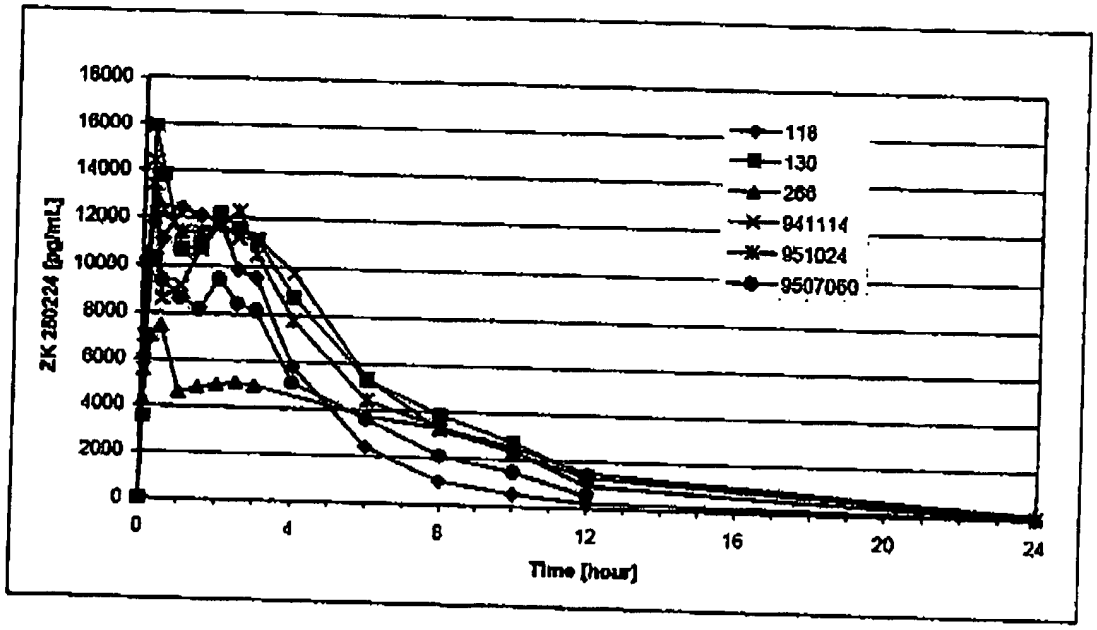
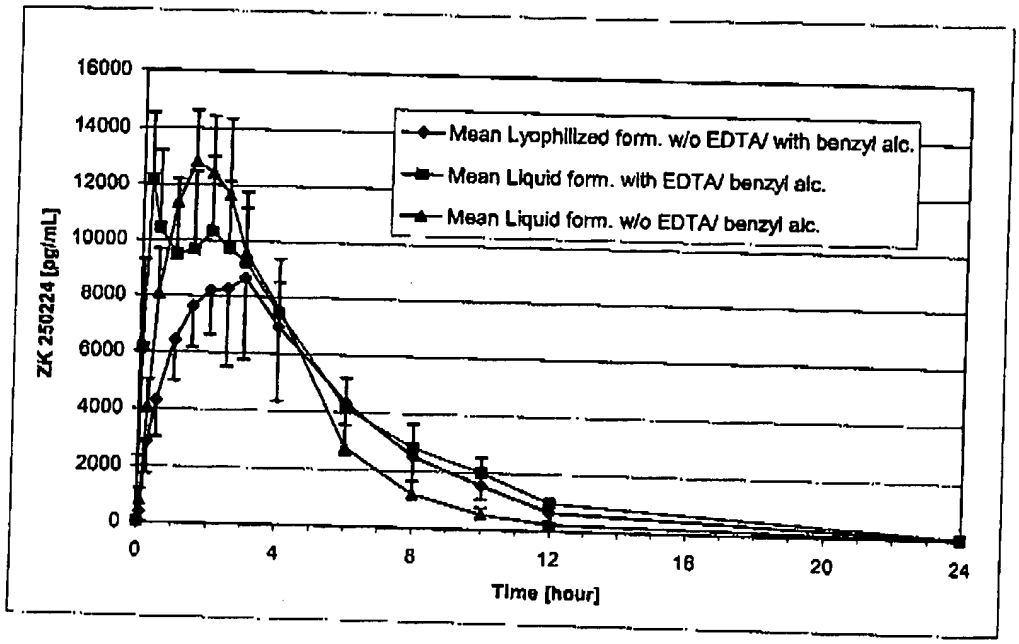


EXHIBIT 3

Mean (± SD) serum concentration time profiles of sargramostim (*i.e.*, ZK 250224) after subcutaneous administration of three different formulations of 20 µg/kg sargramostim to female monkeys (n= 6/ group)



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